Seed and foliar inoculation with *Azospirillum brasilense* associated with nitrogen topdressing rates in wheat crop

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ABSTRACT

The wheat crop (*Triticum aestivum*) is of great importance in human and animal food, being the second largest grain production in the world. However, it is necessary to develop new technologies that increase productivity in a sustainable way, reducing the cost of implementation and operation. Thus, inoculation with growth-promoting bacteria, such as *Azospirillum brasilense*, appears as a viable alternative, but still requires studies for better positioning. The objective of this study was to evaluate the agronomic performance of wheat as a function of the application of *Azospirillum brasilense* via seed and foliar, alone and together, associated with applications of different doses of nitrogen in topdressing. The experiment was conducted in the field at the Federal Institute of Education, Science and Technology of Rio Grande do Sul, Ibirubá campus, in 2020. The experimental design was a randomized block design (DBC) with five replications per treatment, in a two-factor model, with the inoculation factors of *Azospirillum brasilense* (No inoculation; Seed inoculation; Inoculation via foliar and Inoculation via seed + inoculation via foliar) and nitrogen topdressing doses (0, 40, 80 and 120 kg.ha-1) using the wheat cultivar TBIO Ponteiro. The results obtained indicate that inoculation with *Azospirillum brasilense* via seed and foliar, alone and together, did not present significant effects on the variables evaluated, not generating results that make its use feasible, regardless of the form of inoculation, only in the variable number of ears per square meter, which showed an increase with foliar and seed + foliar inoculation. On the other hand, the highest nitrogen doses (80 and 120 kg.ha-1) showed the best results.

Keywords: *Triticum aestivum*, Seed inoculation, Biological nitrogen fixation.

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INTRODUCTION

Wheat (*Triticum aestivum*) is a winter annual plant belonging to the Poaceae family, subfamily Pooideae and the genus *Triticum*. Its origin is believed to be from wild grasses that developed on the banks of the Tigris and Euphrates rivers around 12,000 years BC, after several years of natural and artificial selection the characteristics of the culture that are known today were reached (SCHEEREN et al., 2015).

This cereal has economic importance worldwide, widely used in human food, through flour, bread, pasta, among others. For animal feed it also has its importance, as it is used in various diets in cattle, pigs, poultry, among others. In addition to being one of the main sources of income in winter crops, it provides an important role in crop rotation, and with the straw left on the soil surface, it also contributes to weed control, erosion reduction and improvement of chemical, physical and biological attributes of the soil.

The wheat crop has a great need for nitrogen fertilization to achieve higher yields. According to Prando et al. (2013), nitrogen (N) is the most absorbed nutrient and also the most exported by wheat plants, with a great influence on crop productivity. The most used source of nitrogen fertilization is urea (approximately 45 % of N). However, this nutrient represents a large part of the production cost of wheat crops, because to obtain urea it is necessary to carry out some complex industrial processes (LUDWIG, 2015). Also, this nitrogen source presents an unfavorable characteristic in relation to N loss by volatilization, when applied mainly by haul, without incorporation. The loss can be accentuated the greater the amount of straw and the lack of rain for its incorporation (CANTARELLA et al., 2008).

Knowing the high economic and environmental cost of mineral nitrogen fertilization, alternatives are sought to meet the N demand of the crop, in this context, plant growth-promoting bacteria (BPCP) stand out. These bacteria have the ability to stimulate plant growth in several ways, including: increased nitrate reductase activity when they grow endofitically in plants (CASSÁN et al., 2008), biological N fixation capacity (HUERGO et al., 2008), phosphate solubilization (RODRIGUEZ et al., 2004), and production of phytohormones that induce root growth, improving the absorption of water and nutrients by plants (DOORNBOS et al., 2012). In general, BPCP are capable of stimulating plant growth and development through direct and/or indirect mechanisms, coexisting in an associated way on root surfaces, the rhizosphere and phyllosphere, and also in the internal tissues of plants of different species (HUNGARY et al. 2010).

Among the diazotrophic bacteria, bacteria of the genus *Azospirillum* stand out, which can fix N2 to the plant and thus produce auxins, the main one being indoleacetic acid (IAA), and gibberellins, which stimulate plant growth of the roots, increasing the absorption of water and nutrients (BASHAN et al., 2004). According to Barassi et al. (2008), with the inoculation of

Azospirullum there is an improvement in photosynthetic parameters of the leaves, chlorophyll content, stomatal conductance, increase in the proline content in the roots and shoots, greater biomass production, greater plant height, greater elasticity of the cell wall, improvement in water potential and increase in the water content in the apoplast.

In studies carried out over 20 years, Okon and Labandera-Gonzales (1994) concluded that 60% to 70% of the trials had an increase in production through inoculation, with statistical increases of 5% to 30%. Another study in Argentina, with 273 trials with inoculation of *A. brasilense* in wheat, showed an increase in yield of 256 Kg.ha-1 in 76% of the cases (DÍAZ-ZORITA and FERNANDEZ CANIGIA, 2008). The vast majority of experiments carried out in Brazil and Argentina reported benefits, such as plant growth and/or increased productivity, with the inoculation of *Azospirillum* (CÁSSAN and GARCIA DE SALAMONE, 2008).

According to Döbereiner (1989), in relation to BNF, there is a difference between grass genotypes, thus making it possible to further explore their potential through plant breeding. The lack of response to inoculation of diazotrophic bacteria in grasses, in the vast majority, may be due to the use of inappropriate strains (ROSÁRIO, 2013). Reis et al. (2000), state that the genotype of the plant is essential to achieve BNF benefits, as well as the selection of efficient strains. Competitiveness with other native strains or with the soil microbiota can prevent root colonization by bacteria used as inoculant, which is important for the success of inoculation (REIS, 2007).

Antunes et al. (2017) performing seed inoculation with *Azospirillum brasilense* in maize crop, concluded that it is possible to reduce nitrogen fertilization doses by at least 25% without compromising plant development. In grasses, the transfer of fixed N to the plant occurs slowly and only a small part becomes available to the plant, so the BNF process by these bacteria in association with grasses can only partially supply the plants' nitrogen needs (HUNGARY et al., 2011).

The most commonly used form of inoculation of *A. brasilense* is via seed, although it has the disadvantage that incompatibility with fungicide and insecticide used in seed treatment may occur (HUNGARY et. al., 2007). As an advantage, it has optimization in operationality, since a new application is not necessary, not generating more costs to the producer. Foliar inoculation emerges as an alternative, as it does not present risks with the incompatibility of products in seed treatment, since it is applied later and individually (KAPPES et. al., 2017).

Inoculation via seed requires some care, and must be carried out uniformly, do not leave the seeds exposed to the sun, store in a dry place, sow soon after inoculation or at most in 24 hours, otherwise it is recommended to redo the inoculation. If the seeds are treated with insecticide and/or fungicide, the inoculant should be placed last and be aware of the compatibility of the insecticides and fungicides used in seed treatment (HUNGARY, 2011).

In order to improve operability in the field, the foliar application of the inoculant made by spraying in the vegetative stages of the crop, appears as an option, and has proven to be more practical and efficient, because with the application of inoculant via foliar there is not one of the biggest problems that occurs in inoculation in the seed, which is the incompatibility with insecticides, fungicides and herbicides that can have harmful effects (MOREIRA and SIQUEIRA, 2006).

The present study aims to evaluate the agronomic performance of wheat associated with inoculation of *Azospirillum brasilense* via seed and foliar, isolated and together, with different doses of nitrogen fertilization in topdressing.

MATERIAL AND METHODS

The study was carried out in the experimental area of the Federal Institute of Education, Science and Technology of Rio Grande do Sul (IFRS), Ibirubá campus - RS, with geographic coordinates 28º38'57" S and 53º06'23" W and 452 meters of elevation. The soil is classified as a typical Dystrophic Red Latosol, according to the Brazilian Soil Classification System (SiBCS) (EMBRAPA 2018). According to the Köppen climate classification, the study area is located in a region with a humid subtropical "Cfa" climate, with an average temperature of 19°C and an average annual rainfall of 1826 mm (MORENO, 1961). According to data from the INMET experimental station located at the IFRS Campus Ibirubá, during the sowing period until the harvest of the experiment there was a rainfall of 798.8 mm of rain and the average temperature was 15.5ºC. According to Westphalem (1983) the minimum water requirement for wheat cultivation is 312 mm.

The experimental design used was a randomized block design (DBC) with five replications per treatment involving a two-factor model (4 x 4) with the inoculation factors of *Azospirillum brasilense* (No inoculation; Seed inoculation; Inoculation via foliar and Inoculation via seed + inoculation via foliar) and nitrogen doses in topdressing (0, 40, 80 and 120 kg.ha-1), totaling 80 experimental units. The wheat cultivar used was TBIO Ponteiro, with a final population of 330 plants $^{m-2}$. The experimental units consisted of 9 rows of 3 m in length, with a spacing of 0.17 m between rows, totaling an area of 4.59 m², as shown in Figure 1.

The cropping system used was no-tillage, with soil mobilization only in the sowing row, without future soil disturbance. Soil sampling for further analysis was carried out in the previous crop with soybean crop, considering wheat as the second crop for correction purposes, the yield expectation was 4.5 t.ha-1 and according to the Soil Chemistry and Fertility Commission (2016) the recommended amount of fertilization was 300 Kg.ha-1 of fertilizer of the formula 5-20-20 and there was no need for liming. The recommended N dose was 15 kg.ha-1 at base and 55 kg.ha-1 topdressing, divided into two applications.

In order to eliminate the weeds present in the area and avoid competition for water, light and nutrients with the crop to be implanted, desiccation was carried out using the herbicides Cletodim at a dose of 450 mL.ha-1 b.w., 2,4-D at a dose of 1.5 L.ha-1 b.w. and 0.5 L.ha-1 mineral oil. Sowing took place on June 20, 2020, in a mechanized way, with uniform distribution of seeds in the sowing furrow, at a depth of approximately two cm.

For the treatments with seed inoculation, this was performed before sowing, adding 5 $^{mL.kg-1}$ of liquid inoculant seed composed of a culture of *Azospirillum brasilense* bacteria, of the AbV5 and AbV6 strains, at a concentration of 2.0 x 108 $^{UFC.mL-1}$. For the treatments with foliar inoculant applications, this occurred at the beginning of tillering, corresponding to stage 2 of the Feekes-Large scale, with an electric knapsack sprayer with a flow rate regulated to 365 L.ha-1, considering favorable climatic conditions such as temperature below 20°C, relative humidity above 55-60%, wind speed above 3 km.h-1 and less than 10 km.h-1, seeking to avoid problems with drift, which is the movement of a product in the air during or after application to a different location than planned, and the loss of product efficiency, was using 0.5 L.ha-1 of the same liquid inoculant used via seed.

Nitrogen topdressing was divided into two applications, the first at the beginning of tillering (approximately 40 DAS) and the second at the beginning of stem elongation (approximately 70 DAS), corresponding to stages 2 and 6 of the Feekes-Large scale, respectively. The fertilizer used was urea (45 % nitrogen) and the application was carried out manually, on the surface. The other cultural treatments, such as weed, disease and pest management were carried out according to the Technical Information for wheat and triticale (EMBRAPA 2018), when necessary.

The variables evaluated were emergence (stage 1 of the Feekes-Large scale), and the plants emerged in two linear meters of each experimental unit were counted to estimate the initial stand of the area. When the crop was starting to elongate the stem (stage 6 Feekes-Large scale), the number of tillers per plant was counted in two linear meters per experimental unit and to estimate the number of ears per square meter, the ears of two linear meters of each experimental unit were counted, when all ears were out of the sheaths (stage 10.5 Feekes-Large scale).

In addition, the relative chlorophyll content in the leaves at the beginning of flowering period of the crop (stage 10.5.1 of the Feekes-Large phenological scale) was evaluated, and the relative chlorophyll contents in the adaxial face and central portion of the flag leaf of ten plants per experimental unit were determined, using an electronic chlorophyll content meter (CLOROFILOG CFL1030). At stage 11 of the phenological scale, which corresponds to milky grains, plant height was determined by measuring 10 plants randomly in the experimental unit, with the aid of a graduated tape.

At the moment when the crop reached the physiological maturation stage (stage 11.4 of the Feekes-Large scale), 10 plants per experimental unit (including tillers) were randomly collected, cut at the height of the soil surface. Then, the spikelets of each plant (mother's ear + children's ear) were counted.

After the maturation of the crop, the useful area of 2 meters long by 0.85 meters wide (5 rows), totaling 1.7 m2, was harvested manually, which was threshed in a tractor threshing machine. After cleaning, the mass of the sample was determined on a digital scale with a precision of 0.01 gram, and the sample moisture (U%) was measured using an electronic determiner. From the grain mass obtained in the useful area of the plot, the productivity was obtained, expressed in kg.ha-1, correcting the weight to 13% of moisture and extrapolating the productivity of the plot to hectare.

Regressions were also performed to adjust the degree of polynomial in order to establish the maximum technical and economic efficiency of the productivity variable. Through the equation of degree 2 (y=a±bx±cx2) the mathematical model y= -b1/2b was used, in the estimation of the maximum technical efficiency (MET), for the calculation of the maximum productivity the formula ax^2+bx+c and the formula [(t/w) -b1]/2b2 were used to obtain the maximum economic efficiency (MEE). The t is the value of the input (urea) and w the value of the product (wheat) (DA SILVA et al., 2015), which in this period, the kilogram of urea corresponded to the cost of R$ 3.26 and the amount paid to the producer for the bag of wheat to R$ 81.00.

Through the grain mass used to determine the yield, it was determined on a hectoliteric scale with a capacity of 0.25 L, and then converted to kilograms per hectolitre with the aid of a conversion table that accompanies the equipment. It was also determined by the manual counting of eight replicates of 100 grains weighed on an analytical scale with a precision of 0.001g and after calculating the variance, standard deviation and coefficient of variation.

The collected data were submitted to analysis of variance according to the model of the experimental design and the causes of variation that presented significance by the F test ($p \leq 0.05$) were submitted to complementary procedures according to the responses obtained by the interactions and main effects. For qualitative factors, the Scott-Knott test was used, and for quantitative factors, regression. The software used was Sisvar (FERREIRA, 2019).

RESULTS AND DISCUSSIONS

The average number of emerged plants was 327.9 plants per m2, presenting a satisfactory result, since the seeder was adjusted to distribute 330 plants per m2. Regarding the number of tillers per plant, there was no significant difference between the forms of inoculation (Table 1). Munareto (2016) in his work found an increase in the number of tillers, in two varieties, when the seed was inoculated, while in the other variety there was a decrease in the number of tillers when the seed was inoculated. Barzotto et al. (2018) working with barley, concluded that the number of tillers emitted was higher in the treatments inoculated in the absence of nitrogen fertilization and in the highest dose, which was 120 kg.ha-1. The environmental, nutritional and genetic conditions directly affect the emission of tillers and during this work, the temperatures were raised for tillering. According to Monteiro et al. (2012), the genotype influences the microbial community present in the roots by the difference in the signaling between root and bacteria.

According to table 1, the variable chlorophyll content, a photosynthetic parameter of the leaves, also did not present a statistical difference between the forms of inoculation, unlike the results found by Offeman (2015) and Barassi et al. (2008) who observed increases in chlorophyll content when the seeds were inoculated with *A. brasilense*.

The variable ears per m² showed a statistical difference, where leaf inoculation and seed + leaf inoculation were more efficient in relation to the other forms. On the other hand, even though there was no statistical difference for the number of tillers per plant for the different forms of inoculation, the variable ear per m² presented a higher result for two variables, which can be explained by the fact that these two forms of inoculation had a higher number of viable tillers and were able to generate ears. Unlike Ferreira et al. (2017) and Ferreira et al. (2014) who did not observe a significant difference for this variable when evaluating foliar inoculation in wheat in the Cerrado region.

The number of spikelets per plant was not influenced by the different forms of inoculation, which corroborates the results found by the authors Galindo et al. (2015) and Munareto (2016), who did not observe a statistical difference in the application of *A. brasilense* in wheat for this variable. On the other hand, Munareto (2016), in the experiment in 2015, observed that the association of the forms of inoculation, seed + foliar, presented superior results to the other forms. The joint application of the inoculant has greater potential, due to chemical fertilization not being able to meet the plant's needs in relation to the amount and time of application, unlike the bacterium, where the needs are met continuously until the reproductive phase, which is where the plant most needs N accumulation to change the number of spikelets and the number of grains per ear.

Table 1. tillers per plant, chlorophyll content, number of ears per m2 and spikelets per wheat plant in different forms of inoculation of *A. brasilense* in wheat crop. Ibirubá-RS, 2021.

Form of inoculation	Variable evaluated							
	Children (Plant)		Content of chlorophyll		Ears (m²)		Spikelets (Plant)	
No inoculation	2,2	**ns	46,2	ns	411,5	*b	33,4	ns
Seed inoculation	2,2		47,8		417,4	b	34,3	
Foliar inoculation	2,1		47,6		447,2	a	33,1	
Inoc. Seed + foliar	2,2		49,1		454,0	a	34,9	
Average	2,2		47,7		432,5		33,91	
CV (%)	12,48		7,83		7,5		12,82	

*Means not followed by the same letter in the column differ from each other, by the Scott-Knott test at the 5% level. **ns Not significant by the F test at the 5% level.

It can be seen in table 2 that the yield did not differ between the forms of inoculation, as well as the other characteristics evaluated, plant height, hectolitre weight and weight of one thousand grains. It is pertinent to discuss the variability of responses obtained when working with different genotypes, especially regarding the inoculation of *Azospirillum brasilense*.

Several studies have shown differences in the response of wheat cultivars when inoculated (LEMOS et al., 2013; SALA et al., 2007; SALA et al., 2005). One factor that can interfere with the association is the affinity between bacteria and the plant. Bacteria are attracted to the rhizosphere by the exudates released by plant roots, and use it as an energy source promoting growth (BABALOLA et al., 2010). However, the interaction will depend on factors such as the composition of the exudates (BIANCHET et al., 2013), biotic and abiotic factors of the rhizosphere region (DUTTA; PODILE, 2010) and the competition of *Azospirillum* with native soil diazotrophic bacteria (DIDONET et al., 2000).

In the study carried out by Hungria et al. (2010), significant increases in wheat grain yield of up to 31% were found when they inoculated the seeds with different strains of *A. brasilense*, differently from what was obtained in the present study. For Ferreira (2017), the results in the parameters, hectolitre weight, weight of a thousand grains and productivity did not differ, which corroborates with the results obtained, he also reported that they are due to the fact that the nutrient contents in the soil, especially macronutrients, are in satisfactory conditions, also inferring that the N content could also be adequate by the application of base fertilization and subsequent top fertilization in predecessor crops may have interfered, thus, there were no significant differences between treatments.

Another factor that may have contributed to the lack of difference is that the cultivar used may not have such a positive response in relation to inoculation, since using other cultivars can have more satisfactory results.

Table 2. Plant height, yield, hectolitre weight (PH) and thousand-grain weight (MMG) of wheat in different forms of inoculation of *A. brasilense* in wheat crop. Ibirubá-RS, 2021.

Form of inoculation	Variable evaluated							
	Stature (cm)		Productivity (Kg.Ha-1)		PH (Kg.HL^{-1})		GP (g)	
No inoculation	71,7	*ns	3580,1	ns	77,5	ns	33,1	ns
Seed inoculation	73,1		3416,1		77,5		32,7	
Foliar inoculation	72,1		3467,2		77,7		33,0	
Inoc. Seed + foliar	73,5		3646,1		77,8		33,1	
Average	72,6		3527,4		77,6		33,0	
CV (%)	4,0		11,87		1,06		5,3	

*ns Not significant by the F-test at the 5% level.

Regarding N doses, there was a significant difference for all the variables evaluated, tillers per plant, chlorophyll content, ears per meter² and spikelets per plant, height, productivity, PH, MMG, (Tables 3 and 4).

Table 3. tillers per plant, chlorophyll content, number of ears per m² and spikelets per wheat plant at different doses of nitrogen topdressing fertilization in wheat crop. Ibirubá-RS, 2021.

Dose de N - (Kg ha^{-1})	Variable evaluated							
	Children (Plant)		Chlorophyll Content		Spikes (m²)		Spikelets (Plant)	
0	1,8	c*	44,4	b	384,1	c	29,4	b
40	2,1	b	48,8	a	422,1	b	33,7	a
80	2,3	b	48,7	a	454,4	a	35,5	a
120	2,5	a	48,9	a	469,4	a	37,1	a
Average	2,2		47,7		432,5		33,9	
CV (%)	12,5		7,8		7,5		12,8	

*Means not followed by the same letter in the column differ from each other, by the Scott-Knott test at the 5% level.

Table 4. Plant height, yield, hectolitre weight (PH) and thousand-grain weight (MMG) of wheat at different doses of nitrogen topdressing fertilization in wheat. Ibirubá-RS, 2021.

Dose de N - (Kg.ha^{-1})	Variable evaluated							
	Stature (cm)		Productivity (Kg.Ha-1)		PH (Kg.HL^{-1})		GP (g)	
0	66,1	c*	2831,3	c	78,0	a	33,9	a
40	73,2	b	3595,0	b	78,0	a	33,8	a
80	74,9	a	3704,0	b	77,8	a	33,1	a
120	76,1	a	3979,2	a	76,8	b	31,2	b
Average	72,6		3527,4		77,6		33,0	
CV (%)	4,0		11,87		1,06		5,3	

*Means not followed by the same letter in the column differ from each other, by the Scott-Knott test at the 5% level.

The equations of the N doses mentioned in the sequence obtained significance in the ANOVA to perform the regression analysis. The tillers per plant showed superior results with the increase in N rates (Figure 2A), where the doses of 40 and 80 kg.ha-1 already showed good results, 2.1 and 2.3 tillers per plant, respectively, and the dose of 120 kg.ha-1, presented the highest number of tillers, 2.5 per plant. This can be explained by the fact that this variable is determined by the availability of N in the early stages of crop development (MALAVOLTA, 2006). The results found by Ludwig (2015) corroborate this study, where with higher doses of N a greater number of children was reached. For Barzotto et al. (2018), the dose that provided the highest number of tillers in the barley crop was 62 kg.ha-1 of N for non-inoculated seeds and 43 kg.ha-1 of N for inoculated seeds. Applications of N between emergence and emission of the 3rd leaf allow greater tiller emission and applications on the 7th leaf reduce mortality and postpone tiller senescence, increasing the number of fertile stalks and wheat yield (BREDEMEIER; MUNDSTOCK, 2001).

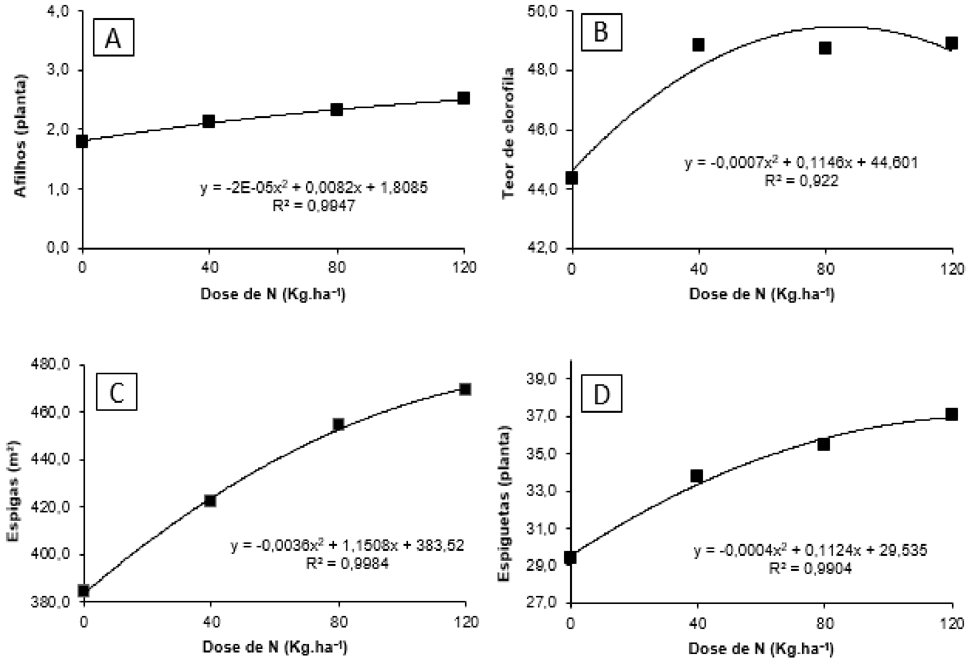
The chlorophyll content showed superior results with the increase in N doses (Figure 2B), the doses 40, 80 and 120 kg.ha-1, with values of 48.8, 48.7 and 48.9 respectively, were higher than the control, which was 44.4. In this sense, Offemann (2015) in his work also concluded that with higher doses of N, leaves with higher chlorophyll levels were obtained. The determination of the chlorophyll content in the leaves shows how the nitrogen nutrition of the plants is, since the main symptom of N deficiency is the yellowing of the leaves (ARGENTA et al. 2001).

Regarding the number of ears, following the trend of tillers, there was an increase in the number of ears as the N dose increased, up to the dose of 80 kg.ha-1, which did not differ from the dose of 120 kg.ha-1 (Figure 2C), with 454.4 and 469.4 ears per m², respectively. This result corroborates that of Marchetti et al. (2001), who obtained an increase in the number of ears with the increase in the doses of N. Barzotto et al. (2018) also concluded that with doses of 80 and 120 kg.ha-1 of N they obtained a higher number of ears in the barley crop.

The number of spikelets per plant showed superior results with the application of N, regardless of the dose (Figure 2D). However, Teixeira Filho et al. (2008) did not observe a significant effect of N doses (0, 30, 60, 90, 120 and 150 kg.ha-1) for the variables number of spikes and number of spikelets per plant.

The variables tiller per plant, ears per m² and spikelets per plant are yield components directly or indirectly determined by the availability of N in the soil, since these enable the accumulation of amino acids, enzymes and proteins in the tissues, which are distributed in the plant, allowing the development of new tissues and accumulated preferentially in the grains (MALAVOLTA, 2006).

Figure 2. A – Number of tillers per plant; B – chlorophyll content; C – number of ears per m²; D – number of spikelets per plant at different doses of nitrogen top dressing fertilization in wheat crop. Ibirubá-RS, 2021



For the variable height (Figure 3A), the doses of 80 and 120 Kg.ha-1 of N were the ones with the highest height. A similar result was obtained by Zagonel et. al. (2002) who observed that according to the increase in N doses, there is also an increase in plant height. Ferreira et al. (2017) also found an increase in the height of wheat plants, since the supply of nitrogen promotes stem elongation and an increase in the number of leaves and tillers. On the other hand, attention should be paid to some cultivars, the greater the height of the plant, the greater the possibility of lodging, a fact that was not observed in the experiment.

Regarding productivity (Figure 3B), the dose of 120 Kg.ha-1 of N was the one that presented the highest productivity. This result is similar to that found by Teixeira Filho (2008), who achieved the maximum productivity of wheat with N doses of 120 Kg.ha-1. On the other hand, Pettinelli Neto et. al. (2002) did not observe an effect of N application on yield increase, due to the supply of N by the predecessor crop, soybean, for several years. The largest increments are obtained in the first 40 kg applied, where the difference for the control without application was 763.8 kg.ha-1, thus highlighting how limiting this element is for the crop (WIETHÖLTER, 2011). For the dose of 40 kg of N $^{ha-1}$, the increase was 19.1 kg of grains for each $^{kg-1}$ of N applied, when compared to the dose of 0 N. For the doses of 80 and 120 kg of N $^{ha-1}$, the increase was only 2.7 and 6.9 kg of grains for each $^{kg-1}$ of N, respectively.

Calculating the maximum technical efficiency, it was obtained values that with 118.2 Kg.ha-1 of N the maximum productivity was reached, which was 3938.7 Kg.ha-1. But calculating the maximum economic efficiency, it was obtained that to be economically viable 117.96 Kg.ha-1 of N should be applied to achieve greater profit.

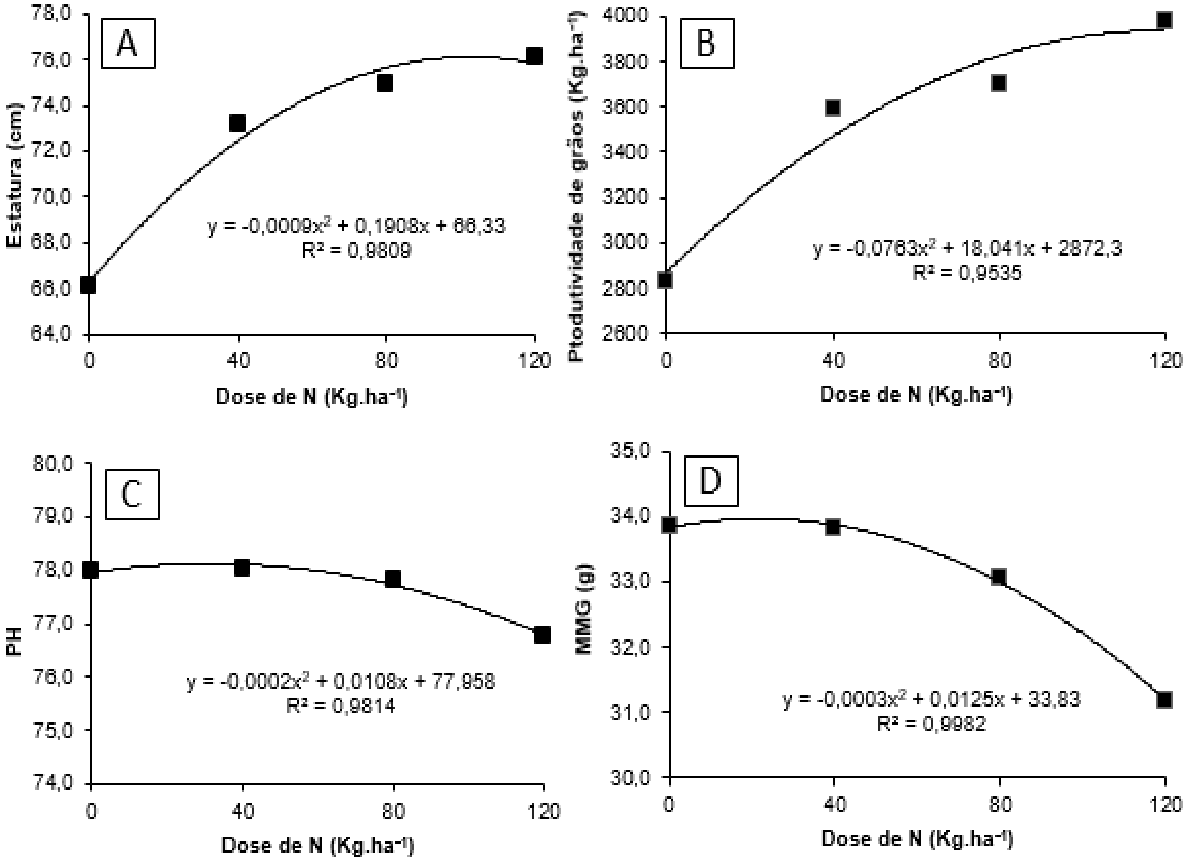
The PH value is used as a measure of wheat commercialization, and indirectly expresses the quality of grains. It is known that the higher the PH value, the greater the acceptance and appreciation of the product in the market (MAZZUCO et al., 2002). The value of 78 kg.hL-1 is used as a reference, that is, PH equal to or above this value, wheat with the highest commercial value is considered. In the determination of the PH, several characteristics of the grain are associated, such as shape, texture of the seed coat, size, mass, and also the presence of impurities (straw, soil). Very low PH values may indicate the occurrence of problems in the crop, which affect the filling and quality of the grain (GUARIENTI, 1993), such as excessive rainfall, for example.

The hectolitre weight had a decreasing value with the increase in N doses (Figure 3C), therefore having a higher value when the N doses were lower or zero (0, 40 and 80 Kg.ha-1) and a lower value with the highest dose (120 Kg.ha-1) as well as Trindade et. al. (2006) and Frizzone et al. (1996), who observed this decrease in hectolitre weight with the increase in nitrogen fertilization. One hypothesis for this reduction is the increase in photoassimilate competition that occurs in grains where high doses of N are applied, also reducing the PH. Sangoi et al. (2007) observed an inverse relationship between protein content in grains at the time of harvest and grain yield, where the most productive cultivar was the one with the lowest protein content, and the cultivar with the highest protein content was the one with the lowest yield. This is due to the greater energy expenditure that the plant has to form protein, which can compromise the accumulation of carbohydrates (SOUZA et al., 2004).

The weight of one thousand grains also showed decreasing values with the increase of N doses (Figure 3D), so the doses 0, 40 and 80 Kg.ha-1 were more efficient in relation to the dose of 120 Kg.ha-1 for this variable, which can be explained according to the results obtained by Teixeira Filho (2008) where he observed that with the increase in the number of grains per ear, increased the competition of photoassimilates within the ear and nutrients, thus reducing the mass of the grains. On the other hand, Barzotto et al. (2018) analyzing the mass of one hundred grains in the barley crop, found results different from those of this work, they concluded that, in inoculated seeds, with the increase in N doses there was an increase in the mass of 100 grains.

The higher doses of N had more tillers per plant and also more ears (fertile tillers), normally the grains from tillers receive less photoassimilated at the time of partitioning and end up filling less. So, having more grains from tillers at a dose of 120 Kg.ha-1 of N, the mass of a thousand grains can be reduced. This result corroborates with Barbieri et al. (2013), who observed that plant tillering is a determining factor for the mass of a thousand grains, that is, plants that develop more tillers have greater intraspecific competition, resulting in a lower mass of one thousand grains. Thus, a plant with a smaller number of tillers is better able to increase the mass of a thousand grains, which can be seen in this work.

Figure 3. A - Plant height; B – grain productivity; C – hectolitre weight (PH); D - weight of one thousand grains (MMG) of wheat at different doses of nitrogen fertilization in wheat crop. Ibirubá-RS, 2021.



CONCLUSIONS

The forms of application of *Azospirillum brasilense* via seed and foliar, isolated and together, did not influence the variables analyzed. Only for the variable ears per m2 there were increments when inoculating via foliar and seed + foliar in relation to the other application options, this result was superior in two forms of inoculation, which can be explained by the fact that these two variables had the highest number of viable tillers and generated ears.

For the cultivar used, the inoculation with *A. brasilense* did not present results that justify and enable its use, regardless of the form of inoculation, and there were no scenarios that brought good yield increases, corresponding to the amounts invested in the use of bacteria of the genus *Azospirillum.*

In the application of nitrogen in the form of urea, mainly in large doses (80 and 120 kg.ha-1) there was an increase in productivity, height, relative chlorophyll content in the leaves, tillers per plant, ears per m2 and spikelets per plant, the higher the dose used, the higher the level of productivity obtained as a response of the plant, where they presented more tillers per plant and also more ears (fertile tillers).

The MMG and PH variables obtained reductions due to the increase in N doses, in the case of MMG the present work presented decreasing results , which can be explained by the increase in the number of grains per ear, which caused an increase in the competition of photoassimilates within the ear and nutrients, thus reducing the grain mass. In the fact of PH, the hypothesis for this reduction is the increase in the competition of photoassimilates that occurs in grains where high doses of N are applied, also reducing the PH, which can be seen in this work.

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